# Acute and Chronic Toxicity of Two Formulations of the Pyrethroid Pesticide Deltamethrin to an Amphipod, Sand Shrimp and Lobster Larvae

W.L. Fairchild, K.G. Doe, P.M. Jackman, J.T. Arsenault,
J.G. Aubé, M. Losier and A.M. Cook

Oceans and Science Branch
Fisheries and Oceans Canada
Gulf Fisheries Centre
343 Université Avenue
Moncton, NB
E1C 9B6

2010

Canadian Technical Report of
Fisheries and Aquatic Sciences 2876

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

**Canadian Technical Report of Fisheries and Aquatic Sciences**

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

**Rapport technique canadien des sciences halieutiques et aquatiques**

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques*.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of
Fisheries and Aquatic Sciences 2876

2010

# ACUTE AND CHRONIC TOXICITY OF TWO FORMULATIONS OF THE PYRETHROID PESTICIDE DELTAMETHRIN TO AN AMPHIPOD, SAND SHRIMP AND LOBSTER LARVAE

by

Wayne L. Fairchild[1], Ken G. Doe[2], Paula M. Jackman[2],
Jacqueline T. Arsenault[1], Jamie G. Aubé[2], Mélanie Losier[2] and Art M. Cook[2]

[1]Fisheries and Oceans Canada, Gulf Fisheries Centre,
343 Université Avenue, Moncton, NB, E1C 9B6
[2]Environment Canada, Science and Technology Branch,
443 Antonine-Maillet Avenue, Moncton, NB, E1A 3E9

E-mail: wayne.fairchild@dfo-mpo.gc.ca

© Her Majesty the Queen in Right of Canada, 2010.
Cat. No. Fs 97-6/2876E ISSN 0706-6457

Correct citation for this publication:
Wayne Fairchild, Ken Doe, Paula Jackman, Jacqueline Arsenault, Jamie Aubé, Mélanie Losier and Art Cook. 2010. Acute and chronic toxicity of two formulations of the pyrethroid pesticide deltamethrin to an amphipod, sand shrimp and lobster larvae. Can. Tech. Rep. Fish. Aquat. Sci. 2876: vi + 34 p.

**TABLE OF CONTENTS**

TABLE OF CONTENTS ..... iii
ABSTRACT ..... iv
RÉSUMÉ ..... v
PREFACE ..... vi
INTRODUCTION ..... 1
MATERIALS AND METHODS ..... 1
  GENERAL SECTION ..... 1
  AMERICAN LOBSTER ..... 4
  MARINE AMPHIPOD ..... 5
  SAND SHRIMP ..... 6
RESULTS ..... 7
  GENERAL SECTION ..... 7
  AMERICAN LOBSTER ..... 8
  MARINE AMPHIPOD ..... 8
  SAND SHRIMP ..... 8
DISCUSSION ..... 8
ACKNOWLEDGEMENTS ..... 10
REFERENCES ..... 10
FIGURES ..... 13
TABLES ..... 15
APPENDICES ..... 18

**ABSTRACT**

Pyrethroid insecticides are among the most toxic insecticides known, and marine crustaceans are generally more sensitive to pyrethroids than marine fish. Among the pyrethroid insecticides, deltamethrin is often the most toxic to crustaceans. Two formulated deltamethrin products are in use in Atlantic Canada. The agricultural formulation is called Decis which is applied to various crops and has potential to enter aquatic habitats via spray drift or runoff. The aquaculture formulation is called AlphaMax, which is applied directly to skirted salmon sea cages to kill sea lice, and is then subsequently released to the estuarine or coastal marine environment. Our objectives were to use marine crustaceans to measure acute toxicity of these two deltamethrin formulations in sea water using conventional 96-hr toxicity test methods, 1-hr pulse exposures and chronic 14- to 16-day exposures. The marine organisms chosen for this study were: *Homarus americanus*, the American lobster (a decapod crustacean); *Crangon septemspinosa*, the sand shrimp (a decapod crustacean); and *Eohaustorius estuarius*, a marine amphipod (an amphipod crustacean). Toxicity results showed that stage III lobster larvae had acute 96-hr $LC_{50}$ (lethal concentration) values for formulated deltamethrin between 3.74 to 4.92 ng/L. Stage IV (post larvae) had 96-hr $LC_{50}$ of 28.2 ng/L. Stage III lobster larvae given a 1-hr pulse exposure followed by 16 days in clean water, had an $LC_{50}$ of 36.5 ng/L. Chronic exposure of stage III lobster larvae for 16 days had an $LC_{50}$ of 4.45 ng/L. The amphipod *E. estuarius* had acute 96-hr $LC_{50}$ values for formulated deltamethrin between 1.66 to 7.99 ng/L. $EC_{50}$ (effective concentration) values for the same tests were from <0.32 to <3.2 ng/L. *E. estuarius* given a 1-hr pulse exposure followed by 95 hrs in clean water had an $LC_{50}$ of 13.3 ng/L, and an $EC_{50}$ of 5.52 ng/L. When *E. estuarius* was given a 48-hr pulse followed by 48 hrs in clean water the $LC_{50}$ was approximately 0.32 with an $EC_{50}$ of <0.032 ng/L. Sand shrimp had acute 96-hr $LC_{50}$ for formulated deltamethrin from 27.4 ng/L for Decis to 45.3 ng/L for AlphaMax. Sand shrimp given a 1-hr pulse exposure followed by 95 hrs in clean water had an $LC_{50}$ of 142 ng/L. Chronic exposure of sand shrimp for 14 days had $LC_{50}$ values from 15.1 (Decis) to 23.8 ng/L (AlphaMax), with $EC_{50}$ (inhibition of growth) from 10.4 (Decis) to >32 ng/L (AlphaMax).

**RÉSUMÉ**

Les insecticides à base de pyréthroïdes comptent parmi les insecticides les plus toxiques connus. Les crustacés marins sont généralement plus sensibles aux pyréthroïdes que les poissons marins. Parmi les insecticides à base de pyréthroïdes, la deltaméthrine est souvent le composé le plus toxique pour les crustacés. Deux produits formulés de deltaméthrine sont en usage au Canada atlantique. La formule agricole, appelée Decis, lorsque appliquée sur diverses cultures, peut entrer dans les habitats aquatiques par entraînement ou ruissellement. La formule aquacole, appelée AlphaMax, est appliquée directement dans les cages à saumon, mouillées en mer et entourées par des jupes, pour tuer le pou de poisson, puis est évacuée dans le milieu estuarien ou côtier. Nos objectifs étaient d'utiliser des crustacés marins pour mesurer la toxicité aiguë de ces deux formules de deltaméthrine en eau de mer en utilisant des méthodes classiques d'essais de toxicité de 96 h, des expositions de courte durée de 1 h et des expositions chroniques de 14 à 16 j. Nous avons choisi pour cette étude les organismes marins suivants : *Homarus americanus*, le homard d'Amérique (un crustacé décapode); *Crangon septemspinosa*, la crevette de sable (un crustacé décapode); et *Eohaustorius estuarius*, un amphipode marin (un crustacé amphipode). Les résultats des essais de toxicité révèlent que la $CL_{50}$ (toxicité aiguë sur 96 h) des produits formulés de la deltaméthrine pour les larves de homard de stade III se situait entre 3,74 et 4,92 ng/L, alors que la $CL_{50}$ – 96 h pour les postlarves (stade IV) se chiffrait à 28,2 ng/L. La $CL_{50}$ pour les larves de stade III confrontées à une exposition de courte durée de 1 h suivie d'un séjour de 16 j en eau propre était de 36,5 ng/L. La $CL_{50}$ suite à une exposition chronique de larves de stade III pendant 16 j était de 4,45 ng/L. La $CL_{50}$ (toxicité aiguë sur 96 h) des produits formulés de la deltaméthrine pour l'amphipode *E. estuarius* allait de 1,66 à 7.99 ng/L. Les valeurs de la $CE_{50}$ (concentration effectif) pour les mêmes essais allaient de $< 0,32$ à $< 3,2$ ng/L. La $CL_{50}$ pour *E. estuarius* confronté à une exposition de courte durée de 1 h suivie d'un séjour de 95 h en eau propre était de 13,3 ng/L et la $CE_{50}$, de 5,52 ng/L. Lorsque *E. estuarius* a été confronté à une exposition de 48 h suivie d'un séjour de 48 h en eau propre, la $CL_{50}$ se chiffrait à environ 0,32 et la $CE_{50}$, à $< 0,032$ ng/L. La $CL_{50}$ (toxicité aiguë sur 96 h) des produits formulés de la deltaméthrine pour la crevette de sable allait de 27,4 ng/L pour Decis à 45,3 ng/L pour AlphaMax. La $CL_{50}$ pour la crevette de sable confrontée à une exposition de courte durée de 1 h suivie d'un séjour de 95 h en eau propre était de 142 ng/L. L'exposition chronique de la crevette de sable pendant 14 j s'est soldée par des valeurs de la $CL_{50}$ allant de 15,1 (Decis) à 23,8 ng/L (AlphaMax) et de la $CE_{50}$ (inhibition de croissance), de 10,4 (Decis) à $> 32$ ng/L (AlphaMax).

## PREFACE

This technical report has been prepared to make available in a timely fashion the results from a series of toxicity tests conducted over the past few years, and particularly the test results from the summer and fall of 2009. The original impetus for this work was potential for off site movement of pyrethroid insecticides from agricultural applications. This was expanded in 2009 to include a pyrethroid formulation relevant to aquaculture practices. Three species of crustaceans were exposed to two formulations (Decis and AlphaMax) of the pyrethroid pesticide deltamethrin. The toxicity results are pertinent to on-going discussions and evaluations of the use of pyrethroids in the treatment of sea lice in Atlantic salmon aquaculture. These results will also provide information for planning further toxicity work on deltamethrin related to its use pattern in salmon net pens in the Bay of Fundy. Some of the analytical chemistry for verification of nominal concentrations was unavailable at the time of publication. This is noted in the relevant appendices.

# INTRODUCTION

Pyrethroid insecticides are among the most toxic insecticides known (Smith and Stratton 1986). They are synthetic analogues of natural pyrethrins that have low mammalian toxicity and greater environmental stability than the natural pyrethrins (Haya 1989, NRCC 1986). Current trends show that agriculture and urban pesticide users are choosing pyrethroid insecticides more frequently, and are opting to use more potent members of the pyrethroid insecticide class (Weston *et al.* 2004, Trimble *et al.* 2009). Marine crustaceans are generally more sensitive to pyrethroids than marine fish (Clark *et al.* 1989), and among the pyrethroids, deltamethrin is often the most toxic to crustaceans in comparative tests (Haya 1989).

Two formulated deltamethrin products are in use in Atlantic Canada (Environment Canada 2004). The agricultural formulation is called Decis, which when used in coastal areas such as in PEI, there is a possibility of exposure of estuarine animals through spray drift and runoff from sprayed fields. The aquaculture formulation is called AlphaMax, which is applied directly to skirted salmon sea cages to kill sea lice, and is then subsequently released to the estuarine or coastal marine environment. Pyrethroids are acutely toxic to estuarine animals in laboratory tests at concentrations at and below 0.01 µg/L; having toxicity at these concentrations places them in a category labeled "supertoxic" chemicals (Clark *et al.* 1989). For perspective, 1 µg/L is one part per billion. Toxicologically significant concentrations then are very low given that 0.01 µg/L mentioned above is 10 parts per trillion. The Canadian Water quality guideline for deltamethrin for the protection of aquatic life in fresh water is 0.0004 µg/L (CCME 1999), below 1 part per trillion.

Our objectives were to use marine crustaceans to measure acute toxicity of deltamethrin formulations in sea water using conventional 96-hr toxicity test methods, 1-hr pulse exposures and chronic and sublethal toxicity tests. The marine organisms chosen for this study were: *Homarus americanus*, the American lobster (a decapod crustacean); *Crangon septemspinosa*, the sand shrimp (a decapod crustacean); and *Eohaustorius estuarius*, a marine amphipod (an amphipod crustacean). These organisms are relevant to the study of pesticide toxicity at environmentally relevant concentrations for the marine environment of Atlantic Canada.

# MATERIALS AND METHODS

## GENERAL SECTION

### Toxicity Test Methods

All toxicity tests were conducted at the Environment Canada Atlantic Laboratory for Environmental Testing (ALET) laboratory in Moncton, NB. This lab is accredited to ISO 17025 standards by the Canadian Association for Laboratory Accreditation (CALA). All testing followed the basic principles outlined in ISO17025.

### Test Chemical - Deltamethrin

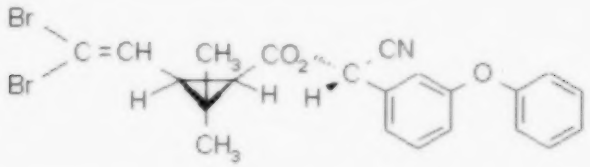
The active ingredient tested in this study was deltamethrin.

Chemical description: (S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2,-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate (Environment Canada 2004)

Emperical formula: $C_{22}H_{19}Br_2NO_3$ (CCME 1999)

CAS number: 52918-63-5 (CCME 1999)

Structural diagram of a deltamethrin molecule:



At 25°C, deltamethrin has water solubility of less than 0.2 ug/L, a vapour pressure of 1.24 x 10-5 mPa, and log $K_{ow}$ 4.6 (Tomlin 2006). UV absorption may cause cis-trans isomerization, splitting of ester bond or loss of bromine (Tomlin 2006).

Toxicity tests were conducted with two formulated products, Decis (used in agriculture) and AlphaMax (used in aquaculture). The Decis formulation was in a liquid state and came from a lot labeled 0171950 Feb 2007 of a 5EC insecticide, PCPA Registration number 22478 (Bayer). The Decis formulation indicated 50g deltamethrin per litre which is 5% active ingredient. The formulation was stored in the dark at 20°C. The AlphaMax formulation was in a liquid state and came from a lot labeled E107A10 (PHARMAQ AS, Oslo, Norway, http://www.pharmaq.no/). The AlphaMax formulation indicated 10g deltamethrin per litre which is 1% active ingredient. The formulation was stored in the dark at 20°C.

**Chemical Confirmation of test concentration**

Water samples were extracted for confirmation of nominal concentrations using a method based on EPA method 508 where pesticides are extracted from the water sample into an organic solvent (methylene chloride) and concentrated (Munch 1995), in this case, by a factor of 8000 times. In some exposure instances, the target concentrations of the pesticide studied were well below the published standard Method Detection Limit (MDL) (i.e. Level of Quantitation) for this procedure (Munch 1995). With additional efforts of sample extract clean-up using silica gel column chromatography, these low determinations were achieved by analyzing the extracts on a Gas Chromatography, Electron Capture Detector (GC-ECD). This technique combined with a large sample volume of 4 liters allowed for Level of Quantitation of 0.33 ng/L. All Quality Assurance/Quality Control steps met the prescribed criteria for the analysis methods used. Method blanks (n=12) displayed no responses and method spike recoveries for deltamethrin at the 5 ng/L level (n=12) ranged from 81-107%. As for the procedural surrogate recoveries for the submitted media samples, these ranged from 72-116%. In addition, 11 of the sample extracts were re-analysed on the GC-ECD and all were within a factor of two of the original value.

**Sea Water Quality**

The source of our seawater used as dilution and control water was the Wharf at Pointe-du-Chêne, NB, in Shediac Bay, on the Northumberland Strait (not an industrialized area). Water was pumped into a 7500 L polyethylene tank (reserved for water alone) and trucked to Moncton, NB. It was pumped into a 30,000 L fiberglass tank through a 40 µm strainer. There was no contact with zinc, copper, or other known toxic materials. Here it was held at approximately 10 - 15° C until used. Water was continuously recirculated and strained through 5 µm water filters. New water was obtained every 4 to 6 weeks, on average. In the spring, this water supply had a salinity of

approximately 26 parts per thousand, and Instant Ocean sea salts were added to bring salinity to 28 parts per thousand for tests with echinoids, polychaetes, certain amphipods, and other organisms. This was done at least a day prior to use. By mid-late summer, the natural salinity was usually around 28 parts per thousand, and remained at this level at least into November. No sea salts were added once the water reached a salinity of 28 parts per thousand. This water has been used to raise polychaetes and amphipods, and to hold sea urchins and marine fish for up to 6 months with no noticeable harmful effects.

Dilution sea water was sampled and received in the Environment Canada chemical analytical laboratory 03-Jun-2009 as Envirodat Project AT0378. Measured variables included $NH_3$/N, Total Suspended Solids (TSS), specific conductivity, pH, alkalinity (Gran and Total), colour, dissolved oxygen, and 21 elements by ICP analysis. A list of variables and the methods used for measurement are given in Table 1. During toxicity tests, temperature range (°C), dissolved oxygen range (mg/L), pH range and salinity (ppt) were measured at intervals given below. To avoid potential cross contamination of test solutions during measurements taken on test solutions, probes were not inserted into the test solutions, instead sub-samples were withdrawn for measurement in small pre-cleaned glass beakers. Test solution pH was measured using an Accumet AB15 pH meter with a combination glass bulb pH electrode. Dissolved oxygen was measured using a YSI Model 58 DO meter with a YSI Model 5905 BOD probe. Temperature was measured using a hand held glass thermometer containing alcohol. Salinity was measured using a Reichert model 13104190 temperature compensated optical refractometer.

**Statistical Analysis of data**

Statistical methods generally followed the procedures recommended in the Environment Canada "Guidance document on statistical methods for environmental toxicity tests", EPS 1/RM/46 (Environment Canada, 2005). The $LC_{50}$ is an estimate of the true median lethal concentration of a test material for a species at the specified exposure time. To provide an indication of the variability of the test population, the $LC_{50}$ estimate was provided with 95% confidence limits. To provide a realistic value for the $LC_{50}$ the most appropriate statistical method must be selected. Following the guidance provided by Stephan (1977) and Environment Canada (2005), we used the following statistical methods in order of preference. For tests with two partial mortalities the probit analysis method was preferred. If probit analyis could not be performed on the data, the Spearman-Karber method was used (the test data must include 0 and 100% mortalities, and at least one partial response). In the Spearman-Karber technique an alpha value, the percentage of data trimming used, must be indicated. Generally, no trimming was selected ($\alpha = 0$). If there were no partial effects (all test organisms died at one concentration and none died at the next lower concentration) binomial calculations were performed. All calculations were based on nominal concentrations.

Mortality in early larval of crustaceans is common in toxicity tests using species such as American lobster. Therefore with the tests using larval lobsters, if mortality occurred in the control (at a level that still permitted a valid test) Abbott's correction was applied to correct for this mortality. This was performed using the CETIS software (Tidepool 2005). The IC25 (EC25) for growth inhibition was calculated using nonlinear regression analysis on CETIS software (Tidepool 2005). Individual tests and any deviations are noted in the appendices with the respective results table for each species

and chemical tested.

**AMERICAN LOBSTER,** *Homarus americanus*

**Acute 96hr exposure**

At the Shippagan Marine Centre, Shippagan, NB, lobster larvae (Fig. 1) were reared from the eggs of adult female lobsters collected in the Gulf of St. Lawrence as part of a lobster stock enhancement program (Comeau 2006). In a genetic study of lobster populations along the east coast of North America, Kenchington *et al.* (2009) found a relatively homogenous population to the north, centered in the Gulf of St. Lawrence. The only exception to homogeneity found in the Gulf of St. Lawrence being an anomalously diverse population sampled near Val Comeau, NB.

Lobster larvae (usually freshly hatched stage I larvae) were transferred into 17 L plastic buckets made of food grade polyethylene, ¾ filled with fresh aquarium sea water (source Shippagan Bay on the Gulf of St. Lawrence). The buckets were then placed at the bottom of a 180 L cylindrical Nalgene tank (71 cm dia. x 45 cm H) a quarter filled with aquarium seawater to act as a temperature bath. Covers with a temperature probe and air stone threaded through two pre-drilled holes were then placed over each bucket. The air stone lines were connected to an air pump powered by a power supply plugged into the vehicle. The bubbling intensity of each air stone was then set to provide oxygenation and to set up a gentle upwelling current to keep the larvae moving in the bucket to help minimize contact between larvae and reduce cannibalism.

A temperature probe was placed in the water of the holding tank and monitored throughout the approximately 4 hour trip to Moncton, NB. During transport, the water temperature in the bath was gradually adjusted from the aquarium seawater temperature in Shippagan, to the seawater temperature at the Environment Canada laboratory in Moncton. If required, a few ice packs were added to the transport tank water to bring its temperature down a few degrees. Temperature differences between facilities were usually less than 5 °C. Lobster larvae obtained as stage I were held in the lab until they reached stage III or IV depending on the exposure design. An approximately 7 day acclimation period under the same conditions as the test was a normal part of the procedure. The holding tank volume was approximately 150 L and a minimum of 20 L fresh water was added daily. There were no visible signs of stress in the organisms. Lobster larvae were fed a combination of live *Artemia* (Salt Creek, Select Brand *Artemia* eggs), dry food (Salt Creek, brine shrimp flakes) and frozen artemia (Hikari, Kyorin Co. Ltd.) and appeared healthy.

Tests were conducted under static conditions with no renewal for acute 96-hr tests. Gentle aeration was provided through glass pipettes giving approximately 100 bubbles per minute. Test vessels were made of glass and were 1 L empty, fill volume was 900 mL. Water quality measurements for temperature, DO, pH and salinity were taken daily for each test concentration in the acute 96 hr tests. The number of replicates for both the control and the treatment in the acute test was 10 to 15. The number of organisms per replicate in both the control and treatment in the acute test was 1. Because we used water miscible in-use pesticide formulations, no solvents were used to aid in preparation of the test solutions. There was no positive control employed for the acute tests. Fluorescent lighting during the test was 100-500 lux on a 16:8 light:dark cycle. All

lobster larval tests were fed every day. Each jar received 5 mg of dry lobster food, 5 mg frozen brine shrimp and 100 live artemia as stage III.

A summary of analytical methods for comparing nominal and measured concentrations are given above. Nominal test concentrations for Decis and AlphaMax during 96-hr acute test are given in the respective test results summaries in the appendix. The nominal concentrations tested across all tests conducted ranged from 0 to 32 ng/L as deltamethrin.

**Deviations for 1 hr pulse exposure**

The nominal concentrations tested ranged from 0 to 1000 ng/L as deltamethrin. For 1-hour pulse exposures the test organism was exposed to the treatment solution for only one hour then transferred to clean water for the remainder of the exposure period. Nominal test concentrations for Decis and AlphaMax during 1-hr pulse exposure tests are given in the respective test results summaries in the appendix.

**Deviations for Chronic exposure**

The nominal concentrations tested across all tests conducted ranged from 0 to 10 ng/L as deltamethrin. Chronic tests were of approximately 16 days duration, ending 9 days after lobster larvae reached stage IV. Water renewal was done three times a week, and there were 25 replicates. For water quality, temperature was measured daily, while DO, pH and salinity were measured three times a week for each test concentration Nominal test concentrations for Decis and AlphaMax during the chronic tests are given in the respective test results summaries in the appendix.

**MARINE AMPHIPOD** *Eohaustorius estuarius*

**Acute 96 hr exposure**

Acute 96-hr exposure followed the reference toxicant procedure in Environment Canada Biological test method EPS 1/RM/26 (Environment Canada 1992). *Eohaustorius estuarius* of 3 – 5 mm size (Fig. 2) were purchased from Northwest Aquatic Sciences (NAS Associates, Inc., Newport, Oregon). They were field collected from Lower Yaquina Bay, Oregon, USA. A minimum 2 day acclimation period under the same conditions as the test was a normal part of the procedure. To reach test conditions acclimation rates of $\leq 3°C$ per day and $\leq 5$ ppt salinity per day were used. *E. estuarius* were not fed and were healthy as observed mortality during shipping and holding was < *5%*. There were no visible signs of stress in the organisms.

Tests were conducted under static conditions with no renewal for acute 96-hr tests. No aeration was provided. Test vessels were made of glass and were 1 L empty, fill volume was 900 mL. Water quality measurements were taken daily for temperature, and at t=0, 48 and 96 hours for pH, DO and salinity for each test concentration. The number of replicates for both the control and the treatment in the acute test was 1. The number of organisms per replicate in both the control and treatment in the acute test was 10. Because we used water miscible in-use pesticide formulations, no solvents were used to aid in preparation of the test solutions. Cadmium was used as a positive control at concentrations of Cd of 19.6, 11.0, 6.13, 3.43, 1.96, 1.10, 0.613 and 0 mg/L. Fluorescent lighting during the test was 100-500 lux on a 16:8 light:dark cycle.

A summary of analytical methods for comparing nominal and measured concentrations are given above. Nominal test concentrations for Decis and AlphaMax during 96-hr acute test are given in the respective test results summaries in the appendix.

The nominal concentrations tested across all tests conducted ranged from 0 to 10,000 ng/L, as deltamethrin.

**Deviations for 1 hr and 48 hr pulse exposure**

The nominal concentrations tested across all tests conducted ranged from 0 to 100 ng/L, as deltamethrin. Test organisms were exposed to the treatment concentrations for defined period (1 or 48 hours) then transferred to clean water for the remainder of the exposure period. Nominal test concentrations for Decis and AlphaMax during the 1-hr and 48-hr pulse exposure tests are given in the respective test results summaries in the appendix.

**SAND SHRIMP** *Crangon septemspinosa*

**Acute 96 hr exposure**

Acute tests of 96-hr exposure followed methods described in Burridge and Haya (1993). *Crangon septemspinosa*, sand shrimp (Fig. 3), were field collected using D-frame dip nets (0.5 mm mesh) from sandy areas of shallow beach habitat off of the Côte-à-Fabien campsite on the shores of Kouchibouguac Bay in Kouchibouguac National Park, NB (46° 50.224' N 064° 56.036' W, GPS Map datum NAD83). Sand shrimp were sorted on site in white trays and the smallest available were transferred into 17 L plastic buckets ¾ filled with fresh sea water from the collection site. The buckets were then placed at the bottom of a 180 L cylindrical Nalgene tank (71 cm dia. x 45 cm H) a quarter filled with sea water to act as a temperature bath. Covers with a temperature probe and air stone threaded through two pre-drilled holes were then placed over each bucket. The air stone lines were connected to an air pump powered by a power supply plugged into the vehicle. The bubbling intensity of each air stone was then set to provide oxygenation and to set up a gentle upwelling current to keep the larvae moving in the bucket to help minimize contact between larvae and reduce cannibalism.

A temperature probe was placed in the water of the holding tank and monitored throughout the approximately 1.5 hour trip to Moncton, NB. During transport, the water temperature in the bath was gradually adjusted from the seawater temperature in Kouchibouguac, to the seawater temperature at the Environment Canada laboratory in Moncton. If required, a few ice packs were added to the transport tank water to bring its temperature down a few degrees throughout the trip.

At test initiation, individual sand shrimp had dry weight of 2-4 mg. A minimum 2 day acclimation period under the same conditions as the test was a normal part of the procedure. There were no visible signs of stress in the organisms. Sand shrimp were fed frozen brine shrimp (Hikari, Kyorin Co. Ltd.) and were healthy as no mortality was observed. Tests were conducted under static conditions with a renewal rate of three times a week. Gentle aeration was provided through glass pipettes giving approximately 100 bubbles per minute. Test vessels were made of glass and were 1 L empty, fill volume was 950 mL. Water quality measurement were taken daily for each test concentration in the acute 96-hr tests. The number of replicates for both the control and the treatment in the acute test was 10. The number of organisms per replicate in both the control and treatment in the acute test was 1. Because we used water miscible in-use pesticide formulations, no solvents were used to aid in preparation of the test solutions. There was no positive control employed for the acute tests. Fluorescent lighting during the test was 100-500 lux on a 16:8 light:dark cycle.

A summary of analytical methods for comparing nominal and measured concentrations are given above. Nominal test concentrations for Decis and AlphaMax during 96-hr acute test are given in the respective test results summaries in the appendix. The nominal concentrations tested across all tests conducted ranged from 0 to 320 ng/L as deltamethrin.

**Deviations for 1 hr pulse exposure**

The nominal concentrations tested across all tests conducted ranged from 0 to 100 ng/L as deltamethrin. For 1-hr pulse exposures the test organism was exposed to the treatment solution for only one hour then transferred to clean water for the remainder of the exposure period. Nominal test concentrations for Decis and AlphaMax during the 1-hr pulse exposure test are given in the respective test results summaries in the appendix.

**Deviations for Chronic exposure**

The nominal concentrations tested across all tests conducted ranged from 0 to 320 ng/L, as deltamethrin. The duration of the chronic exposure test was 14 days. There were 20-30 replicates in both the control (dilution water) and treatments. For water quality, temperature was measured daily, while DO, pH and salinity were measured three times a week for each test concentration. Organism dry weights were determined at the end of the chronic assays. Test organisms were rinsed and transferred to labeled pre-weighed trays, then dried at 60±5°C for a minimum of 24 hours. The weight was determined to the nearest 0.01 mg. The organisms were returned to the ovens for a minimum of 1 hour and then reweighed. This was repeated until a constant weight of within 0.05 mg was achieved. Nominal test concentrations for Decis and AlphaMax during the chronic exposure test are given in the respective test results summaries in the appendix.

## RESULTS

### GENERAL SECTION

**Chemical Confirmation of test concentration**

Results of chemical analyses for confirmation of test solutions are reported in Table C of the respective results summaries for each toxicity test in the Appendices. Most of the measured concentrations of the deltamethrin exposure media for these toxicity tests were similar to nominal values, but 10% (5 out of 52) displayed concentrations that exceeded 2 times the nominal or expected concentration values for the assays. However, the biological response in the 5 tests that were in exceedance, matched that expected from the low nominal or expected concentration. Because of this low biological response at these sampling times, we believe the measured values to be erroneous, however, these exceedances cannot be explained at this time. In most cases aged media analyzed for this study, displayed significant decline in concentrations of the pesticide after 48 hours. Many samples had verified concentration at or below the Level of Quantitation. All negative control samples (n=13) submitted for determination of pesticides displayed less than Levels of Quantitation except for one sample which offered a measurable level which was confirmed with a second analysis.

**Sea Water Quality**

Sea water analysis results for dilution water sampled June 3, 2009 are presented in Table 1. Temperature range (°C), dissolved oxygen range (mg/L), pH range and salinity (ppt) are reported for each toxicity test in Table A of the respective results summaries for each toxicity test in the Appendix.

**AMERICAN LOBSTER** *Homarus americanus*

Stage III lobster larvae had acute 96-hr $LC_{50}$ values for formulated deltamethrin between 3.74 to 4.92 ng/L (N = 3). Stage IV (post larvae) had 96-hr $LC_{50}$ of 28.2 ng/L. Results for the two formulations over three trials were within a range of 1.2 ng/L for stage III, 96-hr $LC_{50}$ (Table 2). Stage III lobster larvae given a 1-hr pulse exposure followed by 16 days in clean water had an $LC_{50}$ of 36.5 ng/L. Chronic exposure of stage III larvae for 16 days had an $LC_{50}$ of 4.45 ng/L (Table 3).

**MARINE AMPHIPOD** *Eohaustorius estuarius*

*E. estuarius* had acute 96-hr $LC_{50}$ values for formulated deltamethrin between 1.66 to 7.99 ng/L. $EC_{50}$ values for the same tests were from <0.32 to <3.2 ng/L. Results for the two formulations fell within these ranges (Table 2). *E. estuarius* given a 1-hr pulse exposure followed by 95 hr in clean water had an $LC_{50}$ of 13.1 ng/L and an $EC_{50}$ of 5.52 ng/L. When given a 48-hr pulse followed by 48 hrs in clean water the $LC_{50}$ was not calculable with an $EC_{50}$ of <0.032 ng/L (Table 2).

**SAND SHRIMP** *Crangon septemspinosa*

*C. septemspinosa* had acute 96-hr $LC_{50}$ for formulated deltamethrin from 27.4 ng/L for Decis to 45.3 ng/L for AlphaMax. *C. septimspinosa* given a 1-hr pulse exposure followed by 95 hrs in clean water had an $LC_{50}$ of 142 ng/L (Table 2). Chronic exposure of sand shrimp for 14 days had $LC_{50}$ values from 15.1 to 23.8 ng/L, with IC25 for growth inhibition (also called EC25) from 10.4 to >32 ng/L (Table 3).

## DISCUSSION

Marine crustaceans are generally more sensitive to pyrethroids than marine fish (Clark *et al.* 1989), and the same pattern is apparent for freshwater crustaceans and fish (Smith and Stratton 1986). Among the pyrethroids, deltamethrin is often the most toxic to crustaceans and fish in comparative tests because of the presence of the dibromovinyl group in the molecule (Haya 1989). Pyrethroids with the alpha cyano group such as deltamethrin are more toxic than the corresponding pyrethroids without, e.g. cypermethrin compared with permethrin, which is less toxic to fish (Haya 1989). Our data confirm the extreme toxicity of pyrethroids to marine crustaceans (Clark *et al* 1989), and are in the same range as past studies on lobster with deltamethrin (Zitko *et al.* 1979) and other pyrethroids (McLeese *et al.* 1980). No literature values for deltamethrin could be found for our other test species (*Eohaustorius estuarius* and *Crangon septemspinosa*).

Interesting to note is the sensitivity of the marine amphipod *Eohaustorius estuarius*. Even as a 1-hour pulse exposure the $LC_{50}$ was 13.1 ng/L and an $EC_{50}$ of 4.46 ng/L was observed (mortality + immobility). After a 48-hr continuous exposure, all test amphipods were immobilized at 0.032 ng/L ($EC_{50}$ of <0.032 ng/L). No recovery was seen on transfer to clean water. A 1-hour pulse with lobster larvae had an $LC_{50}$ of 36.5 ng/L. Good repetition of 96-hr $LC_{50}$ values for deltamethrin (AlphaMax) and stage III lobster larvae were obtained in two tests from July 7 and 29. The two 96-hr $LC_{50}$ values were only 1 ng/L apart at 3.74 and 4.74 ng/L, with 95% Confidence Limits similar with narrow ranges between 2 and 8 ng/L (Table 2, Appendix 2 and 4).

For larval lobster, water temperatures in our tests were in the 18° to 20° C range which is similar to near shore surface water temperatures in the Southern Gulf of St. Lawrence in summer (Koutitonsky and Bugden 1991). Summer water temperatures in the near shore Bay of Fundy would likely have maxima in the 13° to 14° C range, with potential for highest temperatures in sea surface water layers (Trites and Garrett 1983). Pyrethroids have the potential to be more toxic at lower temperatures (NRCC 1986), although Pahl and Opitz (1999) showed the opposite trend for cypermethrin effects on lobster larvae at 10° and 12° C. Lobster larvae tend to be concentrated in the surface layers of the ocean (top 2-3 m), with later larval stages and postlarvae (stage IV) more highly concentrated near the surface (Ennis 1995). Stage III and IV have been shown to be the early life stages most sensitive to pyrethroids at 15° C (Burridge and Haya 1997).

For a point of reference, recommended treatment concentration for control of sea lice in salmon aquaculture for AlphaMax is 3 µg/L (parts per billion) for 40 minutes. For stage III lobster larvae in the vicinity of net pens, the treatment concentration would have to be diluted about 630 to 800 times to reach the 96-hr $LC_{50}$ value for Stage III lobster, and about 110 times to reach the 96-hr $LC_{50}$ value for Stage IV lobster. For Stage III lobster with 1-h exposure followed by 16 days observation in clean water, the treatment concentration would have to be diluted about 82 times to reach the 1-hr $LC_{50}$ value. For the amphipod *Eohaustorius estuarius*, the treatment concentration would have to be diluted about 1,800 times to reach the 96-hr $LC_{50}$ value, and about 230 times to reach the 1-hr $LC_{50}$ value. The concentrations of deltamethrin causing irreversible immobility to *Eohaustorius estuarius* were generally less than the lowest concentration tested, and further testing is required to establish threshold toxicity values for this sensitive endpoint. For a 1-hr exposure to *Eohaustorius estuarius*, the recommended treatment concentration would have to be diluted about 540 times to reach the 1-hr $EC_{50}$ (immobilization). Sand shrimp were generally less sensitive than the amphipod and the lobster. Based on the study by Ernst *et al.* (2001), it would likely take several hours (estimated at 2 to 4 hours) for the recommended treatment concentration to reach 100 to 1000 times dilution. From the results of our study, we believe that there is risk of exceeding the 1-hr $LC_{50}$ for stage III lobster (36.5 ng/L) and the 1-hr $EC_{50}$ (immobilization)(5.52 ng/L) for the amphipod *Eohaustorius estuarius* for distances of 100 + meters and times of one to several hours post treatment, depending on the site characteristics and timing of water movements. This poses a risk to these crustaceans in the vicinity of operational releases of deltamethrin from salmon net pens. If several net pens were treated in the same area on the same day, the risk of adverse effects would be increased.

Because of its high log $K_{ow}$ of 4.6 (Tomlin 2006), deltamethrin would be expected to absorb onto suspended particles and eventually be incorporated into bottom sediments. The study by Westin *et al.* (2004) showed high toxicity of deltamethrin in freshwater sediment toxicity tests to amphipods. Muir *et al.* (1985) found sediments to be the major sink for deltamethrin in experimentally treated freshwater ponds, and showed that chironomid larvae exposed for 48 hrs accumulated radiolabelled deltamethrin from sediments 360 days after treatment. We believe further studies of deltamethrin incorporated into marine sediments with sediment dwelling amphipods will be needed to determine risk of this pesticide to sediment dwelling invertebrates. The toxicity of deltamethrin absorbed onto suspended particles to water column organisms is unknown and should also be investigated.

## ACKNOWLEDGMENTS

Funding for this study comes from the Fisheries and Oceans, Canada National Pesticide Research Fund (NPRF), the Environment Canada, Pesticide Science Fund (PSF), and the Fisheries and Oceans Canada, Fisheries Collaborative Research Program (FSCP, Michel Comeau, Martin Mallet and Homarus Inc.). Further support was provided through Environment Canada and Fisheries and Oceans Canada on-going programs. Wild sand shrimp were collected with cooperation from Kouchibouguac National Park, Parks Canada. Lobster larvae were provided with cooperation from Martin Mallet of Homarus Inc., and the facilities of the Shippagan Marine Centre, Shippagan, NB. Dr. Mike Beattie of New Brunswick Department of Agriculture and Aquaculture, Province of New Brunswick, provided the AlphaMax formulation used in the toxicity tests. Lobster life cycle figure provided by Marc Lanteigne, DFO, Moncton, NB. Technical and professional staff at Environment Canada Moncton conducted many transfers, measurements and counts, as well as chemical characterization of the assay mediums for this study; Fabiola Akaishi, Nicole Arsenault, Megan Bauer, Tria Clare, Dan Greenberg, Charles Hopper, Dale Hughes and Chantal Theriault. Dr. Les Burridge and Bill Ernst provided valuable review comments on a draft of this manuscript.

## REFERENCES

Burridge, L.E. and K. Haya. 1993. The lethality of ivermectin, a potential agent for treatment of salmonids against sea lice, to the shrimp *Crangon septemspinosa*. Aquaculture 117: 9- 14.

Burridge, L.E. and K. Haya. 1997. The lethality of pyrethrins to larvae and postlarvae of the American lobster (*Homarus americanus*). Ecotox. Environ. Safety. 38:150-154.

CCME. 1999. Deltamethrin. Canadian Environmental Quality Guidelines, Canadian council of Ministers of the Environment. Excerpt from Pub. No.1299: 3 p.

Clark, J.R., L.R. Goodman, P.W. Borthwick, J.M. Patrick, Jr., G.M. Cripe, P.M. Moody, J.C. Moore, and E.M. Lores. 1989. Toxicity of pyrethroids to marine invertebrates and fish: a literature review and test results with sediment-sorbed chemicals. Environ. Toxicol. Chem. 8:393-401.

Comeau, M. 2006. Lobster (*Homarus americanus*) enhancement project in the southwestern Gulf of St. Lawrence, Canada. Alaska Crab Stock Enhancement and Rehabilitation: Workshop Proceedings, Alaska Sea Grant College Program, AK-SG-06-04, pp. 71-78.

Ennis, G.P. 1995. Larval and postlarval ecology. *In*: Biology of the Lobster *Homarus americanus*. Edited by J.R. Factor. Academic Press, Inc., San Diego, California, USA. pp. 23-46.

Environment Canada. 1992. Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods. Report EPS 1/RM/26. Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 2004. A compendium of information on pesticides used in Atlantic Canada. Dartmouth, Nova Scotia. EPS-1-AR-04-01. 384 p.

Environment Canada. 2005. Guidance document on statistical methods for environmental toxicity tests. Ottawa, Ontario. EPS 1/RM/46. 170 p.

Ernst, W., P. Jackman, K. Doe, F. Page, G. Julien, K. MacKay, and T. Sutherland. 2001. Dispersion and toxicity to non-target aquatic organisms of pesticides used to treat sea lice on salmon in net pen enclosures. Mar. Pollut. Bull. 42:433-444.

Haya, K. 1989. Toxicity of pyrethroid insecticides to fish. Environ. Toxicol. Chem. 8:381-391.

Kenchington, E.L., G.C. Harding, M.W. Jones and P.A. Prodöhl. 2009. Pleistocene glaciation events shape genetic structure across the range of the American lobster, *Homarus americanus*. Molecular Ecol. 18:1654–1667.

Koutitionsky, V.G. and G.L. Bugden. 1991. The physical oceanography of the Gulf of St. Lawrence: a review with emphasis on the synoptic variability of the motion. *In* The Gulf of St. Lawrence: small ocean or big estuary? Edited by J.-C. Therriault. Can. Spec. Publ. Fish. Aquat. Sci. 113. p. 57-90.

McLeese, D.W., C.D. Metcalfe, and V. Zitko. 1980. Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp. Bull. Environ. Contam. Toxicol. 25:950-955.

Munch, J.W. 1995. Method 508-Determination of chlorinated pesticides in water by gas chromatography with an electron capture detector (Rev 3.1), National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 30 pp.

Muir, D.C.G., G.P. Rawn and N.P. Grift. 1985. Fate of the pyrethroid insecticide deltamethrin in small ponds: a mass balance study. J. Agric. Food Chem. 33:603-609.

NRCC. 1986. Pyrethroids: Their effects on aquatic and terrestrial ecosystems. Environmental Secretariat, National Research Council of Canada. Ottawa, ON. Pub. No. 24376: 303 p.

Pahl, B.C. and H.M. Opitz. 1999. The effects of cypermethrin (Excis) and azamethiphos (Salmosan) on lobster *Homarus americanus* H. Milne Edwards larvae in a laboratory study. Aquaculture Res. 30:655-665.

Smith, T.M. and G.W. Stratton. 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Res. Rev. 97:93-120.

Stephan, C.E. 1977. Methods for calculating an $LC_{50}$. *In* Aquatic toxicology and hazard evaluation. Edited by F.L. Mayer and J.L. Hamelink. Amer. Soc. Testing and Materials, Philadelphia, PA. ASTM STP no. 634. p. 65-84.

Tidepool Scientific Software. 2005. Comprehensive Environmental Toxicity Information System (CETIS), Statistical Software Program.Version1.6. McKinleyville, CA

Tomlin, C.D.S. 2006. The pesticide manual, a world compendium. 14th edition, British Crop Council Production (BCCP), Surrey, U.K. 1344 pp.

Trimble, A.J., D.P. Weston, J.B. Belden and M.J. Lydy. 2009. Identification and evaluation of pyrethroid insecticide mixtures in urban sediments. Environ. Toxicol. Chem. 28:1687-1695.

Trites, R.W. and C.J.R. Garrett. 1983. Physical oceanography of the Quoddy Region. *In* Marine and coastal systems of the Quoddy Region, New Brunswick. Edited by M.L.H. Thomas. Can. Spec. Publ. Fish. Aquat. Sci. 64. p. 9-34.

Weston, D.P., J. You and M.J. Lydy. 2004. Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's Central Valley. Environ. Sci. Toxicol. 38:2752-2759.

Zitko, V., D.W. McLeese, C.D. Metcalfe and W.G. Carson. 1979. Toxicity of permethrin, decamethrin, and related pyrethroids to salmon and lobster. Bull. Environ. Contam. Toxicol. 21:338-343.

## FIGURES

**FIGURE 1: Typical life cycle of the American lobster (*Homarus americanus*) showing stage III (carapace length about 3.25 mm) and stage IV (carapace length about 4.0 mm) individuals. (Image courtesy of Fisheries and Oceans Canada)**

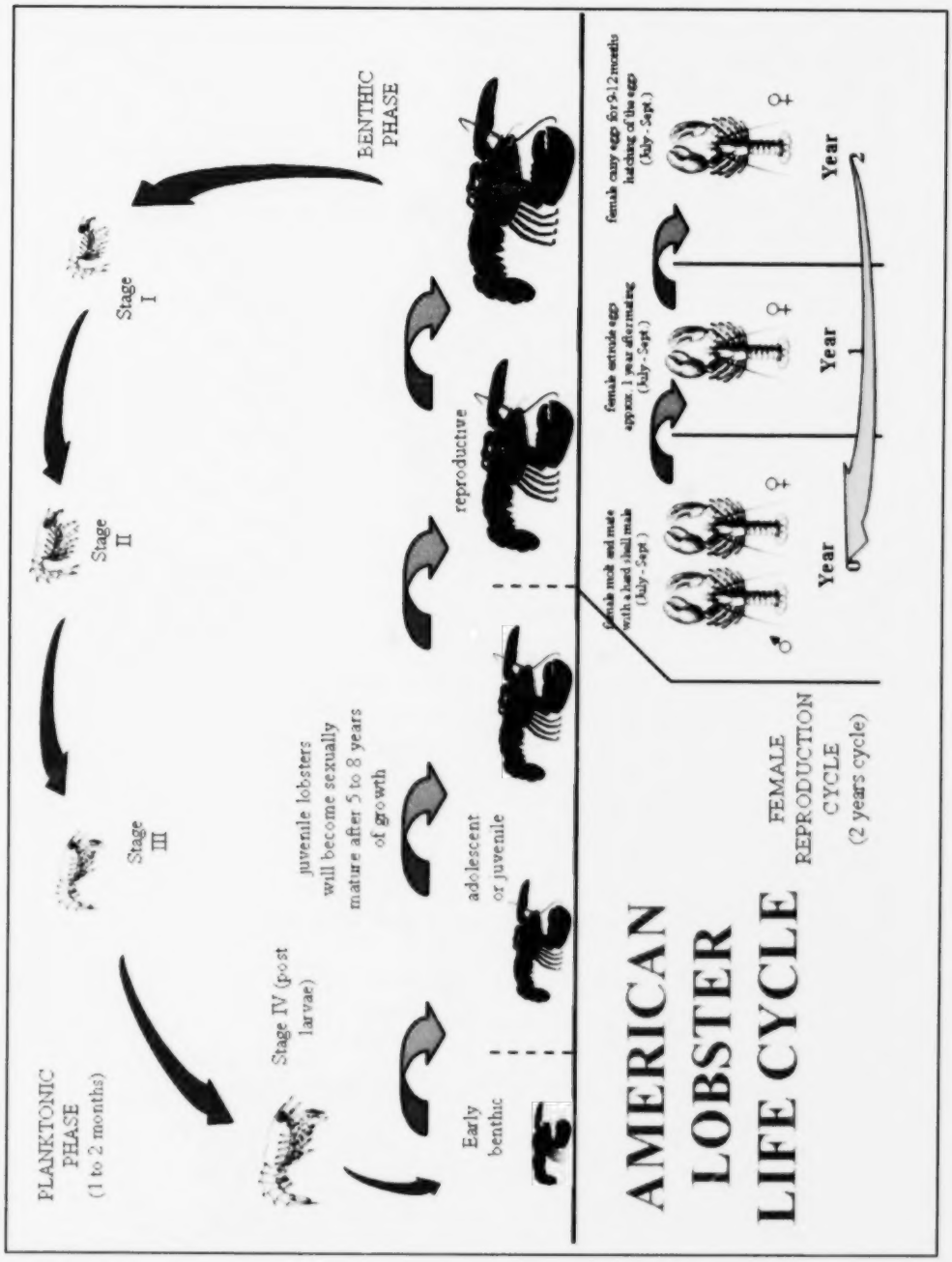

PLANKTONIC PHASE (1 to 2 months)
Stage III
Stage IV (post larvae)
Stage II
Stage I
BENTHIC PHASE
juvenile lobsters will become sexually mature after 5 to 8 years of growth
Early benthic
adolescent or juvenile
reproductive
AMERICAN LOBSTER LIFE CYCLE
FEMALE REPRODUCTION CYCLE (2 years cycle)
female molt and mate with a hard shell male (July - Sept.)
female extrude eggs approx. 1 year afterwards (July - Sept.)
female carry eggs for 9-12 months hatching of the eggs (July - Sept.)
Year 0
Year 1
Year 2

**FIGURE 2: Marine amphipod (*Eohaustorius estuarius*), field collected from lower Yaquina bay, Oregon, USA, large juveniles to adults used in tests are 3 to 5 mm total length. (Picture courtesy of Stewart Yee and Environment Canada)**

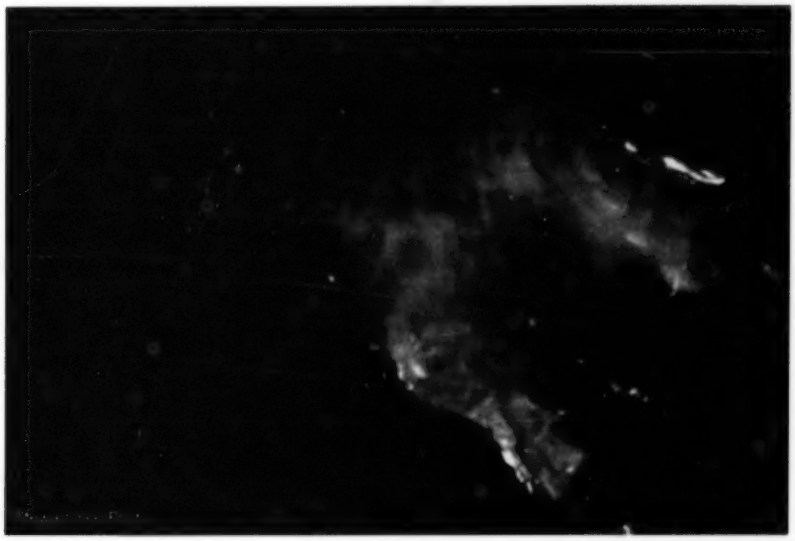
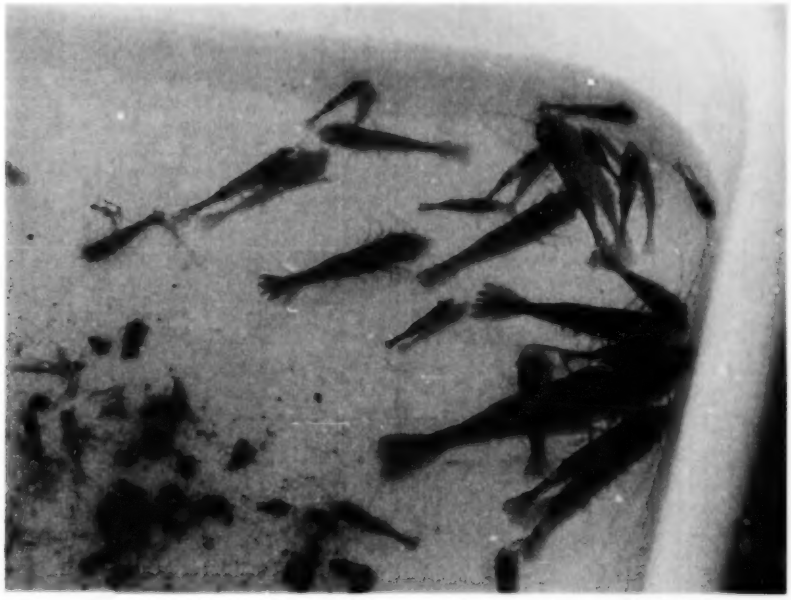


**FIGURE 3: Sand shrimp (*Crangon septemspinosa*), in sorting tray. Sand shrimp were collected from pristine areas in Kouchibouguac National Park. Only the smallest juveniles are used in the toxicity tests measuring growth and survival. Rounded edge of sorting tray is 12 mm thick. (Picture courtesy of Fisheries and Oceans Canada)**



## TABLES

**TABLE 1: Salt Water Analysis of dilution water for toxicity testing**

| Variable | Value | Units | Method |
|---|---|---|---|
| $NH_3/N$ | <0.002 | mg/L | Color phenate FIA |
| TSS | <2.0 | mg/L | Filter gravimetric |
| Al | 20 | µg/L | DA-Plasma ICP-OES |
| Ba | 8 | µg/L | DA-Plasma ICP-OES |
| Be | <1 | µg/L | DA-Plasma ICP-OES |
| Cd | <3 | µg/L | DA-Plasma ICP-OES |
| Cr | <2 | µg/L | DA-Plasma ICP-OES |
| Co | <5 | µg/L | DA-Plasma ICP-OES |
| Cu | <2 | µg/L | DA-Plasma ICP-OES |
| Fe | <0.02 | mg/L | DA-Plasma ICP-OES |
| Pb | <20 | µg/L | DA-Plasma ICP-OES |
| Mn | <2 | µg/L | DA-Plasma ICP-OES |
| Mo | 6 | µg/L | DA-Plasma ICP-OES |
| Ni | <6 | µg/L | DA-Plasma ICP-OES |
| Ag | <2 | µg/L | DA-Plasma ICP-OES |
| Sr | 6400 | µg/L | DA-Plasma ICP-OES |
| Tl | <1 | µg/L | DA-Plasma ICP-OES |
| V | <4 | µg/L | DA-Plasma ICP-OES |
| Zn | <2 | µg/L | DA-Plasma ICP-OES |
| Na | 9472 | mg/L | Atom Emission ICP-OES |
| K | 326.5 | mg/L | Atom Emission ICP-OES |
| Ca | 347.9 | mg/L | Atom Emission ICP-OES |
| Mg | 1069 | mg/L | Atom Emission ICP-OES |
| Spec. cond. | 41500 | µS/cm | PC-titration |
| pH | 7.91 | pH units | Electro PC-titration |
| Alkalinity Gran | 103.8 | mg/L | Electro PC-titration |
| Alkalinity Total | 106.0 | mg/L | Electro PC-titration |
| Colour | <5 | Hazen Units | FIA (Colour-App) |
| Dissolved Oxygen | 90-100 | % saturation | YSI Model 58 DO Meter |

Sea water sampled and received in laboratory 03-Jun-2009. Envirodat Project AT0378.

**TABLE 2: Summary of Acute Test Results with Decis and AlphaMax formulations of deltamethrin.** Concentrations in the table are all given as ng/L (parts per trillion) of active ingredient as nominal values. Details of results found in noted Appendix.

| Start date/ Appendix | Formulation | Species, stage and duration | $LC_{50}$ in ng/L (95% Confidence Limits) | $EC_{50}$ in ng/L |
|---|---|---|---|---|
| 07-Jul-09 1 | Decis | *Homarus americanus* - stage III 96 hour | 4.92 (3.24 – 7.46) | |
| 07- Jul-09 2 | AlphaMax | *Homarus americanus* - stage III 96 hour | 4.74 (2.96 – 7.60) | |
| 14-Jul-09 3 | AlphaMax | *Homarus americanus* - stage IV 96 hour | 28.2 (12.6 – 595) | |
| 29-Jul-09 4 | AlphaMax | *Homarus americanus* - stage III 96 hour | 3.74 (2.27 – 6.22) | |
| 26-Jun-09 5 | Decis | *Eohaustorius estuarius* 96 hour | < 3.2 | < 3.2 |
| 02-Jul-09 6 | Decis | *Eohaustorius estuarius* 96 hour | 7.99 (4.75- 13.4) | <0.32 |
| 04-Jul-09 7 | AlphaMax | *Eohaustorius estuarius* 96 hour | 1.66 (0.29 – 4.78) | <0.32 |
| 14-Aug-09 8 | AlphaMax | *Eohaustorius estuarius* 1 hour pulse + 95 hour clean water | 13.1 (4.77 – 35.8) | 5.52 |
| 17-Aug-09 9 | AlphaMax | *Eohaustorius estuarius* 48 hour pulse+ 48 hour clean water | Approx. 0.32 can not calculate | < 0.032 |
| 27-Oct-09 10 | AlphaMax | *Crangon septemspinosa* 1 hour pulse + 95 hour clean water | 142 (104 – 194) | |
| 27-Oct-09 11 | AlphaMax | *Crangon septemspinosa* 96 hour | 45.3 (28.2 – 73.3) | |
| 3-Nov-09 12 | Decis | *Crangon septemspinosa* 96 hour | 27.4 (16.3 – 47.4) | |

**TABLE 3: Summary of Chronic Test Results with Decis and AlphaMax formulations of deltamethrin.** Please note the concentrations in the table are all given as ng/L (parts per trillion) of active ingredient as nominal values. Results found in noted Appendix.

| Start date/ Appendix | Formulation | Species, stage and duration | LC 50 (ng/L) | IC25 (ng/L) |
|---|---|---|---|---|
| 8-Jul-09 13 | AlphaMax | *Homarus americanus* stage III, 1-hour pulse + 16 day clean water | 36.5 (25.0 – 53.3) | |
| 8-Jul-09 14 | AlphaMax | *Homarus americanus* stage III, 16 day | 4.45 (3.69 – 5.36) | |
| 19-Aug-09 15 | Decis | *Homarus americanus* - stage III, 14 day | Not calc. (40% control mortality) | |
| 31-Aug-07 16 | Decis | *Crangon septemspinosa* 14 day | 15.1 (12.5– 18.1) | 10.4 (3.21 – 17.6) |
| 11-Sep-09 17 | AlphaMax | *Crangon septemspinosa* 14 day | 23.8 (19.1 – 29.7) | >32 |

# APPENDICES

## APPENDIX 1

Chemical - Formulation: Deltamethrin - Decis
Test species: *Homarus americanus* stage III
Duration: 96-hour exposure
Test start date: July 7, 2009
Test end date: July 11, 2009
Nominal test concentrations: 32, 10, 3.2, 1, 0.32 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 4.92 (3.24 – 7.46) calculated using Spearman-Karber analysis ($\alpha$ = 0) using CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 32 | 18.8 – 19.8 | 7.3 – 7.8 | 7.81 – 8.00 | 28 |
| 10 | 18.9 – 20.0 | 7.6 – 8.0 | 7.84 – 8.05 | 28 |
| 3.2 | 18.9 – 20.0 | 7.2 – 8.0 | 7.80 – 8.01 | 28 |
| 1 | 18.9 – 19.9 | 7.7 – 8.0 | 7.86 – 7.97 | 28 |
| 0.32 | 18.7 – 19.9 | 7.5 – 8.0 | 7.83 – 7.96 | 28 |
| 0 | 18.9 – 19.9 | 7.6 – 7.9 | 7.78 – 7.91 | 28 |

Table B

| Test concentration (ng/L) | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|
| 32 | 11/15 | 15/15 | 15/15 | 15/15 |
| 10 | 0/15 | 9/15 | 9/15 | 9/15 |
| 3.2 | 4/15 | 7/15 | 9/15 | 9/15 |
| 1 | 1/15 | 1/15 | 1/15 | 1/15 |
| 0.32 | 1/15 | 1/15 | 1/15 | 1/15 |
| 0 | 1/15 | 2/15 | 2/15 | 2/15 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 32 | 30.8 |
| 3.2 | 5.61 |
| 0.32 | 3.59 |
| 0 | <0.33 |

**APPENDIX 2**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Homarus americanus* stage III
Duration: 96-hour exposure
Test start date: July 7, 2009
Test end date: July 11, 2009
Nominal test concentrations: 32, 10, 3.2, 1, 0.32 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 4.74 (2.96 – 7.60) calculated using Spearman-Karber analysis ($\alpha$ = 0) using CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 32 | 20.0 – 20.3 | 7.6 – 7.8 | 7.90 – 7.97 | 28 |
| 10 | 20.0 – 20.7 | 7.5 – 8.0 | 7.83 – 7.98 | 28 |
| 3.2 | 20.0 – 20.6 | 7.4 – 8.2 | 7.82 – 7.98 | 28 |
| 1 | 20.0 – 20.6 | 7.5 – 8.3 | 7.84 – 7.97 | 28 |
| 0.32 | 20.0 – 20.7 | 7.5 – 7.8 | 7.83– 7.96 | 28 |
| 0 | 20.0 – 20.7 | 7.4 – 8.2 | 7.64 – 7.97 | 28 |

Table B

| Test concentration (ng/L) | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|
| 32 | 13/15 | 15/15 | 15/15 | 15/15 |
| 10 | 5/15 | 10/15 | 10/15 | 10/15 |
| 3.2 | 0/15 | 4/15 | 5/15 | 5/15 |
| 1 | 3/15 | 5/15 | 6/15 | 6/15 |
| 0.32 | 1/15 | 1/15 | 1/15 | 1/15 |
| 0 | 3/15 | 3/15 | 3/15 | 3/15 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 32 | 20.7 |
| 3.2 | 11.0* |
| 0.32 | 42.3* |
| 0 | <0.33 |

*Outside expected values.

**APPENDIX 3**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Homarus americanus* stage IV
Duration: 96-hour exposure
Test start date: July 14, 2009
Test end date: July 18, 2009
Nominal test concentrations: 32, 10, 3.2, 1, 0.32 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 28.2 (12.6 – 595) Calculated using Probit analysis using Toxstats software with correction for control mortality (Abbott's formula).

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 32 | 19.6 – 20.9 | 7.4 – 8.0 | 7.85 – 7.98 | 28 |
| 10 | 19.8 – 20.9 | 7.4 – 8.0 | 7.66 – 7.97 | 28 |
| 3.2 | 19.8 – 20.9 | 7.0 – 7.9 | 7.78 – 7.94 | 28 |
| 1 | 20.0 – 20.9 | 7.0 – 8.0 | 7.77 – 7.99 | 28 |
| 0.32 | 20.0 – 20.9 | 7.3 – 7.8 | 7.81– 7.87 | 28 |
| 0 | 20.0 – 21.0 | 7.4 – 8.2 | 7.81 – 7.89 | 28 |

Table B

| Test concentration (ng/L) | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|
| 32 | 7/10 | 7/10 | 7/10 | 7/10 |
| 10 | 2/10 | 2/10 | 2/10 | 2/10 |
| 3.2 | 2/10 | 2/10 | 3/10 | 3/10 |
| 1 | 0/10 | 0/10 | 0/10 | 0/10 |
| 0.32 | 0/10 | 0/10 | 0/10 | 0/10 |
| 0 | 0/10 | 1/10 | 1/10 | 1/10 |

**APPENDIX 4**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Homarus americanus* stage III
Duration: 96-hour exposure
Test start date: July 29, 2009
Test end date: August 2, 2009
Nominal test concentrations: 32, 10, 3.2, 1, 0.32 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 3.74 (2.27 – 6.22) calculated using probit analysis on Toxstats software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 32 | 19.8 – 21.4 | 7.2 – 7.7 | 7.88 – 7.94 | 28 |
| 10 | 19.8 – 21.3 | 7.4 – 7.8 | 7.91 – 7.98 | 28 |
| 3.2 | 19.9 – 21.2 | 7.4 – 7.7 | 7.88 – 7.94 | 28 |
| 1 | 20.0 – 21.1 | 7.3 – 7.7 | 7.91 – 7.96 | 28 |
| 0.32 | 19.9 – 21.2 | 7.3 – 7.7 | 7.81 – 7.94 | 28 |
| 0 | 19.9 – 21.2 | 7.3 – 7.6 | 7.63 – 7.91 | 28 |

Table B

| Test concentration (ng/L) | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|
| 32 | 13/15 | 15/15 | 15/15 | 15/15 |
| 10 | 5/15 | 9/15 | 10/15 | 11/15 |
| 3.2 | 2/15 | 3/15 | 3/15 | 6/15 |
| 1 | 1/15 | 1/15 | 1/15 | 2/15 |
| 0.32 | 1/15 | 1/15 | 1/15 | 1/15 |
| 0 | 0/15 | 0/15 | 0/15 | 0/15 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 32 | 18.5 |
| 3.2 | 2.2 |
| 0.32 | 0.32 |
| 0 | <0.33 |

**APPENDIX 5**

Chemical - Formulation: Deltamethrin - Decis
Test species: *Eohaustorius estuarius*
Duration: 96-hour exposure
Test start date: June 26, 2009
Test end date: June 30, 2009
Nominal test concentrations:
10,000, 3200, 1000, 320, 100, 32, 10, 3.2 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ <3.2; 96-hour $EC_{50}$ <3.2 ng/L
No statistical calculation methods are applicable to this data.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 10000 | 15.0 – 16.5 | 7.2 – 7.9 | 7.81 – 8.01 | 28-29 |
| 3200 | 15.0 – 16.5 | 6.2 – 7.8 | 7.78 – 8.01 | 28-29 |
| 1000 | 15.0 – 16.5 | 6.6 – 7.9 | 7.83 – 8.00 | 28-29 |
| 320 | 14.9 – 16.5 | 7.1 – 7.7 | 7.84 – 7.99 | 28-29 |
| 100 | 14.9 – 16.5 | 6.8 – 7.8 | 7.80 – 7.98 | 28-29 |
| 32 | 14.9 – 16.5 | 7.0 – 7.9 | 7.80 – 7.98 | 28-29 |
| 10 | 14.9 – 16.5 | 7.1 – 7.8 | 7.78 – 7.97 | 28-29 |
| 3.2 | 14.9 – 16.5 | 7.1 – 7.8 | 7.76 – 7.95 | 28-29 |
| 0 | 14.9 – 16.5 | 7.0 – 7.7 | 7.75 – 7.92 | 28-29 |

Table B

| Test concentration (ng/L) | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed | 96 hour # Immobile/ # Exposed |
|---|---|---|---|---|---|
| 10000 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 3200 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 1000 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 320 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 100 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 32 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 10 | 10/10 | 10/10 | 10/10 | 9/10 | 1/10 |
| 3.2 | 2/10 | 10/10 | 10/10 | 8/10 | 0/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |

I = animal is immobilized, twitching but can not move

**APPENDIX 6**

Chemical - Formulation: Deltamethrin - Decis
Test species: *Eohaustorius estuarius*
Duration: 96-hour exposure
Test start date: July 2, 2009
Test end date: July 6, 2009
Nominal test concentrations: 32, 10, 3.2, 1, 0.32, and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 7.99 (4.75 – 13.4); calculated using Spearman-Karber analysis ($\alpha$ = 0) on Toxstats software
96-hour $EC_{50}$ <0.32 no confidence limits; no statistical calculation methods are applicable.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 32 | 14.7 – 16.0 | 7.5 – 8.0 | 7.80 – 7.89 | 28-29 |
| 10 | 14.7 – 16.0 | 7.4 – 7.9 | 7.78 – 7.89 | 28-29 |
| 3.2 | 14.7 – 16.0 | 7.5 – 7.8 | 7.78 – 7.88 | 28-29 |
| 1 | 14.7 – 16.0 | 7.5 – 8.0 | 7.77 – 7.89 | 28-29 |
| 0.32 | 14.7 – 16.0 | 7.6 – 8.0 | 7.74 – 7.89 | 28-29 |
| 0 | 14.7 – 16.0 | 7.1 – 7.9 | 7.73 – 7.90 | 28-29 |

Table B

| Test concentration (ng/L) | 1 hour # Immobile/ # Exposed | 2 hour # Immobile/ # Exposed | 4 hour # Immobile/ # Exposed | 24 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed | 96 hour # Immobile/ # Exposed |
|---|---|---|---|---|---|---|
| 32 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 10 | 0/10 | 10/10 | 10/10 | 10/10 | 2/10 | 8/10 |
| 3.2 | 0/10 | 10/10 | 10/10 | 10/10 | 4/10 | 5/10 |
| 1 | 0/10 | 2/10 | 10/10 | 10/10 | 1/10 | 9/10 |
| 0.32 | 0/10 | 0/10 | 1/10 | 8/10 | 0/10 | 10/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |

I = animal is immobilized, twitching but can not move

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 10 | 6.91 |
| 1 | 1.64 |
| 0 | 5.98* |

* Outside expected values.

**APPENDIX 7**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Eohaustorius estuarius*
Duration: 96-hour exposure
Test start date: July 4, 2009. Test end date: July 8, 2009
Nominal test concentrations: 100, 32, 10, 3.2, 1, 0.32, and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 1.66 (0.29 – 4.78) calculated using probit analysis on Toxstats software. 96-hour $EC_{50}$ < 0.32 no confidence limits; no statistical calculation methods are applicable to this data.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 100 | 14.8 – 15.7 | 7.4 – 7.7 | 7.95 – 8.05 | 29 |
| 32 | 14.8 – 15.8 | 7.4 – 7.7 | 7.94 – 8.04 | 29 |
| 10 | 14.8 – 15.8 | 7.3 – 7.5 | 7.93 – 8.03 | 29 |
| 3.2 | 14.8 – 15.8 | 7.2 – 7.5 | 7.92 – 7.99 | 29 |
| 1 | 14.8 – 15.5 | 7.2 – 7.5 | 7.92 – 8.01 | 29 |
| 0.32 | 14.8 – 15.7 | 7.1 – 7.5 | 7.90 – 8.00 | 29 |
| 0 | 14.8 – 16.7 | 7.0 – 7.6 | 7.82 – 7.98 | 28-29 |

Table B

| Test concentration (ng/L) | 1 hour # Immobile/ # Exposed | 2 hour # Immobile/ # Exposed | 4 hour # Immobile/ # Exposed | 24 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed | 96 hour # Immobile/ # Exposed |
|---|---|---|---|---|---|---|
| 100 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 32 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 0/10 |
| 10 | 10/10 | 10/10 | 10/10 | 10/10 | 5/10 | 5/10 |
| 3.2 | 3/10 | 8/10 | 10/10 | 10/10 | 7/10 | 3/10 |
| 1 | 0/10 | 0/10 | 8/10 | 9/10 | 4/10 | 3/10 |
| 0.32 | 0/10 | 0/10 | 1/10 | 6/10 | 3/10 | 4/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |

I = animal is immobilized, twitching but can not move

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 10 | 8.11 |
| 1 | 0.98 |
| 0 | <0.33 |

**APPENDIX 8**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Eohaustorius estuarius*
Duration: 1-hour exposure + 95 hour clean water
Test start date: August 14, 2009
Test end date: August 18, 2009
Nominal test concentrations: 100, 32, 10, 3.2, 1, 0.32, and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 13.1 (4.77 – 35.8); calculated using Trimmed Spearman-Karber analysis ($\alpha$ = 47.8 %) on CETIS software. Note the data were not monotonic (they were variable), a high trim had to be used to calculate the $LC_{50}$.
96-hour $EC_{50}$ 5.52 (5.10 – 5.97); calculated using Trimmed Spearman-Karber analysis ($\alpha$ = 4.17 %) on CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 100 | 15.6 – 16.9 | 7.5 – 8.2 | 7.85 – 7.99 | 27-28 |
| 32 | 15.5 – 16.8 | 7.2 – 8.2 | 7.84 – 7.98 | 27-28 |
| 10 | 15.6 – 16.7 | 7.7 – 8.1 | 7.84 – 7.98 | 27-28 |
| 3.2 | 15.4 – 16.7 | 7.7 – 8.2 | 7.82 – 7.97 | 27-28 |
| 1 | 15.6 – 16.8 | 7.2 – 8.0 | 7.75 – 7.95 | 27-28 |
| 0.32 | 15.5 – 16.8 | 7.3 – 7.9 | 7.72 – 7.92 | 27-28 |
| 0 | 15.5 – 17.0 | 7.2 – 8.1 | 7.62 – 7.80 | 27-28 |

Table B

| Test concentration (ng/L) | 1 hour # Immobile/ # Exposed | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed | 96 hour # Immobile/ # Exposed |
|---|---|---|---|---|---|---|
| 100 | 10/10 | 10/10 | 10/10 | 10/10 | 7/10 | 3/10 |
| 32 | 10/10 | 10/10 | 10/10 | 10/10 | 8/10 | 2/10 |
| 10 | 0/10 | 10/10 | 10/10 | 10/10 | 5/10 | 5/10 |
| 3.2 | 0/10 | 4/10 | 3/10 | 2/10 | 1/10 | 0/10 |
| 1 | 0/10 | 4/10 | 2/10 | 0/10 | 2/10 | 1/10 |
| 0.32 | 0/10 | 1/10 | 0/10 | 0/10 | 2/10 | 1/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | 1/10 | 1/10 |

I = animal is immobilized, twitching but can not move

**APPENDIX 9**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Eohaustorius estuarius*
Duration: 48-hour exposure + 48 hour clean water
Test start date: August 17, 2009
Test end date: August 21, 2009
Nominal test concentrations: 3.2, 1, 0.32, 0.10, 0.032 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ approx. 0.32 can not calculate; no statistical calculation methods are applicable for this data.
96-hour $EC_{50}$ <0.032; no statistical calculation methods are applicable for this data.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) | # Dead/ # Exposed | # Immobile/ # Exposed |
|---|---|---|---|---|---|---|
| 3.2 | 15.0 – 16.0 | 7.0 – 8.4 | 7.75 – 7.95 | 28 | 5/10 | 5/10 |
| 1 | 15.0 – 16.1 | 6.9 – 8.4 | 7.68 – 7.93 | 28 | 5/10 | 5/10 |
| 0.32 | 14.9 – 16.2 | 6.8 – 8.3 | 7.63 – 7.93 | 28 | 6/10 | 4/10 |
| 0.1 | 14.9 – 16.2 | 6.5 – 8.3 | 7.62 – 7.93 | 28 | 3/10 | 7/10 |
| 0.032 | 14.9 – 16.3 | 6.7 – 8.2 | 7.72 – 7.92 | 28 | 3/10 | 7/10 |
| 0 | 15.1 – 16.4 | 6.6 – 8.2 | 7.70 – 7.93 | 28 | 0/10 | 0/10 |

Table B

| Test concentration (ng/L) | 24 hour # Immobile/ # Exposed | 48 hour # Immobile/ # Exposed | 72 hour # Immobile/ # Exposed | 96 hour # Dead/ # Exposed | 96 hour # Immobile/ # Exposed |
|---|---|---|---|---|---|
| 3.2 | 10/10 | 10/10 | 10/10 | 5/10 | 5/10 |
| 1 | 10/10 | 10/10 | 10/10 | 5/10 | 5/10 |
| 0.32 | 10/10 | 10/10 | 10/10 | 6/10 | 4/10 |
| 0.1 | 10/10 | 10/10 | 10/10 | 3/10 | 7/10 |
| 0.032 | 10/10 | 10/10 | 10/10 | 3/10 | 7/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |

I = animal is immobilized, twitching but can not move

**APPENDIX 10**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Crangon septemspinosa*
Duration: 1-hour exposure + 95 hour clean water
Test start date: October 27, 2009
Test end date: October 31, 2009
Nominal test concentrations: 1000, 320, 100, 32, 10 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 142 (104 – 194)
Calculated using Spearman-Karber analysis ($\alpha = 0$) in Toxstats software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) | # Dead/ # Exposed |
|---|---|---|---|---|---|
| 1000 | 15.0 – 15.2 | 8.3 – 8.4 | 7.70 – 7.72 | 29 | 10/10 |
| 320 | 15.0 – 15.3 | 8.2 – 8.3 | 7.71 – 7.76 | 29 | 10/10 |
| 100 | 15.1 – 16.1 | 8.1 – 8.4 | 7.70 – 7.86 | 29 | 0/10 |
| 32 | 15.2 – 16.1 | 8.0 – 8.4 | 7.71 – 7.85 | 29 | 2/10 |
| 10 | 15.5 – 15.9 | 7.9 – 8.3 | 7.69 – 7.84 | 29 | 0/10 |
| 0 | 15.4 – 16.0 | 7.9 – 8.3 | 7.67 – 7.78 | 29 | 0/10 |

Table B

| Test concentration (ng/L) | 1 hour # Dead/ # Exposed | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 72 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|---|
| 1000 | 0/10 (9I) | 10/10 | 10/10 | 10/10 | 10/10 |
| 320 | 0/10 (2I) | 10/10 | 10/10 | 10/10 | 10/10 |
| 100 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 32 | 0/10 | 0/10 | 0/10 | 1/10 | 2/10 |
| 10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |

I = animal is immobilized, twitching but can not move

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 1000 | 2009002081* |
| 320 | 2009002080* |
| 3.2 | 2009002079* |
| 1 | 2009002078* |
| 0 | 2009002077* |

* Samples have been taken, data not available at this time.

**APPENDIX 11**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Crangon septemspinosa*
Duration: 96-hour continuous exposure
Test start date: October 27, 2009
Test end date: October 31, 2009
Nominal test concentrations: 100, 32, 10, 3.2, 1.0 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 45.3 (28.2 – 73.3)
Calculated using probit analysis using Toxstats software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) | # Dead/ # Exposed |
|---|---|---|---|---|---|
| 100 | 15.4 – 16.3 | 8.1 – 8.4 | 7.71 – 7.82 | 29 | 9/10 |
| 32 | 15.4 – 16.5 | 7.9 – 8.2 | 7.69 – 7.84 | 29 | 3/10 |
| 10 | 15.4 – 16.5 | 7.8 – 8.3 | 7.68 – 7.81 | 29 | 0/10 |
| 3.2 | 15.4 – 16.4 | 8.0 – 8.2 | 7.69 – 7.80 | 29 | 0/10 |
| 1.0 | 15.5 – 16.4 | 8.0 – 8.2 | 7.69 – 7.82 | 29 | 0/10 |
| 0 | 15.6 – 16.6 | 7.7 – 8.2 | 7.64 – 7.79 | 29 | 0/10 |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 72 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|
| 100 | 6/10 | 7/10 | 9/10 | 9/10 |
| 32 | 2/10 | 2/10 | 2/10 | 3/10 |
| 10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 3.2 | 0/10 | 0/10 | 0/10 | 0/10 |
| 1.0 | 0/10 | 0/10 | 0/10 | 0/10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples |
|---|---|
| 1000 | 2009002081* |
| 320 | 2009002080* |
| 3.2 | 2009002079* |
| 1 | 2009002078* |
| 0 | 2009002077* |

* Samples have been taken, data not available at this time.

**APPENDIX 12**

Chemical - Formulation: Deltamethrin - Decis
Test species: *Crangon septemspinosa*
Duration: 96-hour continuous exposure
Test start date: November 3, 2009
Test end date: November 7, 2009
Nominal test concentrations: 320, 100, 32, 10, 3.2 and 0 ng/L, as deltamethrin.
Result: 96-hour $LC_{50}$ 27.4 (16.3 – 47.4)
Calculated using probit analysis in Toxstats.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 320 | 15.5 – 15.6 | 8.2 – 8.3 | 7.77 – 7.79 | 28 |
| 100 | 15.5 – 15.7 | 8.1 – 8.3 | 7.75 – 7.76 | 28 |
| 32 | 15.5 – 15.9 | 8.1 – 8.4 | 7.73 – 7.84 | 28 |
| 10 | 15.5 – 15.9 | 8.1 – 8.3 | 7.71 – 7.84 | 28 |
| 3.2 | 15.4 – 16.0 | 8.1 – 8.2 | 7.70 – 7.82 | 28 |
| 0 | 16.0 – 16.4 | 8.0 – 8.4 | 7.63 – 7.76 | 28 |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 72 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed |
|---|---|---|---|---|
| 320 | 10/10 | 10/10 | 10/10 | 10/10 |
| 100 | 10/10 | 10/10 | 10/10 | 10/10 |
| 32 | 0/10 | 4/10 | 4/10 | 4/10 |
| 10 | 0/10 | 1/10 | 2/10 | 2/10 |
| 3.2 | 0 /10 | 0 /10 | 0 /10 | 0 /10 |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 |

**APPENDIX 13**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Homarus americanus* stage III
Duration: 1-hour exposure +16 day clean water, 9 days after stage IV
Test start date: July 8, 2009
Test end date: July 24, 2009
Nominal test concentrations: 1000, 100, 10, 1 and 0 ng/L, as deltamethrin.
Result: 16-day $LC_{50}$ 36.5 (25.0 – 53.3) calculated using Spearman-Karber analysis ($\alpha$ = 0) in CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 1000 | 19.5 – 19.8 | 7.8 | 7.87 – 7.92 | 28 |
| 100 | 19.4 – 21.4 | 7.5 – 8.1 | 7.86 – 7.99 | 28 |
| 10 | 19.7 – 21.1 | 7.2 – 8.1 | 7.78 – 7.99 | 28 |
| 1 | 19.6 – 21.2 | 7.2 – 8.1 | 7.81 – 7.93 | 28 |
| 0 | 19.7 – 21.0 | 4.5 – 7.9 | 7.52 – 7.90 | 28 |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed | 7 day # Dead/ # Exposed | 16 day # Dead/ # Exposed | 16 day # stage III/ # stage IV/ # stage V |
|---|---|---|---|---|---|---|
| 1000 | 25/25 | 25/25 | 25/25 | 25/25 | 25/25 | |
| 100 | 22/25 | 23/25 | 23/25 | 23/25 | 23/25 | 0/1/1 |
| 10 | 5/25 | 7/25 | 7/25 | 8/25 | 10/25 | 0/12/3 |
| 1 | 2/25 | 3/25 | 3/25 | 7/25 | 9/25 | 0/12/4 |
| 0 | 0/25 | 0/25 | 4/25 | 7/25 | 10/25 | 0/14/1 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples | Measured test concentration (ng/L) after renewal to clean water t=2 days |
|---|---|---|
| 1000 | 960 | * |
| 100 | 113 | * |
| 10 | 7.54 | <0.33 |
| 1 | 1.25 | <0.33 |
| 0 | <0.33 | <0.33 |

* Samples not taken. Organisms all dead.

**APPENDIX 14**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Homarus americanus* stage III
Duration: 16-day continuous exposure, 9 days after stage IV
Test start date: July 8, 2009
Test end date: July 24, 2009
Nominal test concentrations: 10, 3.2, 1 and 0 ng/L, as deltamethrin.
Result: 16-day $LC_{50}$ 4.45 (3.69 - 5.36) calculated using Spearman-Karber analysis ($\alpha = 0$) in CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 10 | 19.0 – 20.1 | 7.5 – 8.3 | 7.70 – 8.00 | 28 |
| 3.2 | 19.9 – 21.2 | 7.4 – 8.2 | 7.62 – 8.02 | 28 |
| 1 | 19.7 – 21.2 | 7.2 – 8.0 | 7.77 – 7.99 | 28 |
| 0 | 19.9 – 21.2 | 7.2 – 8.0 | 7.64 – 7.93 | 27 – 28 |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed | 7 day # Dead/ # Exposed | 16 day # Dead/ # Exposed | 16 day # stage III/ # stage IV/ # stage V |
|---|---|---|---|---|---|---|
| 10 | 0/25 | 10/25 | 25/25 | 25/25 | 25/25 | |
| 3.2 | 0/25 | 0/25 | 3/25 | 5/25 | 8/25 | 0/15/2 |
| 1 | 0/25 | 0/25 | 1/25 | 2/25 | 2/25 | 0/21/2 |
| 0 | 1/25 | 3/25 | 5/25 | 5/25 | 5/25 | 0/19/0 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples | Measured test concentration (ng/L) t=5 days samples before renewal | Measured test concentration (ng/L) t=5 days samples after renewal |
|---|---|---|---|
| 10 | 7.54 | | 8.91 |
| 3.2 | 2.65 | Interference | 2.96 |
| 1 | 1.25 | Interference | 1.35 |
| 0 | <0.33 | <0.33 | <0.33 |

**APPENDIX 15**

Chemical - Formulation: Deltamethrin - Decis
Test species: *Homarus americanus* stage III
Duration: 14-day continuous exposure, 9 days after stage IV
Test start date: August 19, 2009
Test end date: September 2, 2009
Nominal test concentrations: 10, 3.2, 1 and 0 ng/L, as deltamethrin.
Result: Not calculated (40% control mortality).

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 10 | 20.0 – 21.0 | 7.5 – 7.7 | 7.77 – 7.96 | 27 |
| 3.2 | 19.7 – 21.0 | 7.3 – 7.8 | 7.68 – 7.95 | 27 - 28 |
| 1 | 19.7 – 21.0 | 5.7 – 7.6 | 7.69 – 7.93 | 27 – 28 |
| 0 | 19.8 – 21.0 | 5.4 – 7.8 | 7.49 – 7.94 | 27 – 28 |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed | 7 day # Dead/ # Exposed | 14 day # Dead/ # Exposed | 14 day # stage III/ # stage IV/ # stage V |
|---|---|---|---|---|---|---|
| 10 | 15/25 | 23/25 | 25/25 | 25/25 | 25/25 | |
| 3.2 | 5/25 | 14/25 | 18/25 | 19/25 | 21/25 | 1/3/0 |
| 1 | 3/25 | 10/25 | 10/25 | 15/25 | 21/25 | 0/4/0 |
| 0 | 1/25 | 5/25 | 8/25 | 8/25 | 10/25 | 0/15/0 |

High control mortality

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=0 samples | Measured test concentration (ng/L) t=7 days samples before renewal | Measured test concentration (ng/L) t=7 days samples after renewal |
|---|---|---|---|
| 10 | 10.8 | | |
| 3.2 | 5.29 | 5.23 | 7.21 |
| 1 | 1.21 | 26.8* | 10.3* |
| 0 | <0.33 | <0.33 | <0.33 |

*Outside expected values.

**APPENDIX 16**

Chemical - Formulation: Deltamethrin - Decis
Test species: *Crangon septemspinosa*
Duration: 14-day continuous exposure
Test start date: August 31, 2007. Test end date: September 14, 2007
Nominal test concentrations: 320, 100, 32, 10, 3.2 and 0 ng/L, as deltamethrin.
Result: 14-day $LC_{50}$ 15.1 (12.5 – 18.1) calculated using Spearman-Karber analysis ($\alpha$ = 0) on Toxstats software. 14-day EC25 10.4 (3.21 – 17.6) growth inhibition calculated using nonlinear regression analysis on CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 32 | 14.7 -15.7 | 7.7 – 8.4 | 7.85 – 8.09 | 28 |
| 10 | 14.7 – 15.9 | 7.7 – 8.5 | 7.82 – 8.08 | 28 |
| 3.2 | 14.8 – 15.9 | 7.9 – 8.4 | 7.87 – 8.07 | 28 |
| 1 | 14.9– 16.0 | 8.0 – 8.4 | 7.88 – 8.06 | 28-29 |
| 0.32 | 14.9 – 16.0 | 7.7 – 8.3 | 7.80 – 8.16 | 28-29 |
| 0 | 15.1 – 16.7 | 7.9 – 8.2 | 7.86 – 8.10 | 28 |
| Initial | NA | NA | NA | NA |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed | 7 day # Dead/ # Exposed | 14 day # Dead/ # Exposed | 14 day Dry weight mg |
|---|---|---|---|---|---|---|
| 32 | 0/20 | 0/20 | 20/20 | 20/20 | 20/20 | NA |
| 10 | 0/20 | 0/20 | 2/20 | 3/20 | 3/20 | 2.62 ± 1.01 |
| 3.2 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 2.97 ± 0.76 |
| 1 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 2.90 ± 1.07 |
| 0.32 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 3.33 ± 1.22 |
| 0 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 3.71± 1.55 |
| Initial | NA | NA | NA | NA | NA | 2.38 ± 0.82 |

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=3 samples Fresh solutions | Measured test concentration (ng/L) t=5 days samples before renewal |
|---|---|---|
| 10 | 2007001659* | 2007001737* |
| 1 | 2007001658* | 2007001736* |
| 0 | 2007001657* | 2007001735* |

* Samples have been taken, data not available at this time.

**APPENDIX 17**

Chemical - Formulation: Deltamethrin - AlphaMax
Test species: *Crangon septemspinosa*
Duration: 14-day continuous exposure
Test start date: September 11, 2009. Test end date: September 25, 2009
Nominal test concentrations: 100, 32, 10, 3.2, 1 and 0 ng/L, as deltamethrin.
Result: 14-day $LC_{50}$ 23.8 (19.1 – 29.7); calculated using Spearman-Karber analysis ($\alpha$ = 0) on CETIS software. 14-day EC25 growth >32; calculated using Linear Interpolation on CETIS software.

Table A

| Test concentration (ng/L) | Temperature range (°C) | DO range (mg/L) | pH range (pH units) | Salinity (ppt) |
|---|---|---|---|---|
| 100 | 15.5 – 16.3 | 3.9 – 8.4 | 7.74 – 7.91 | 27 – 28 |
| 32 | 15.8 – 17.1 | 4.9 – 8.3 | 7.65 – 7.91 | 27 – 28 |
| 10 | 15.8 – 17.1 | 7.7 – 8.3 | 7.64 – 7.88 | 27 – 28 |
| 3.2 | 15.8 – 16.8 | 7.9 – 8.4 | 7.59 – 7.92 | 27 – 28 |
| 1 | 15.6 – 16.5 | 7.9 – 8.2 | 7.57 – 7.88 | 27 – 28 |
| 0 | 15.8 – 16.8 | 8.0 – 8.2 | 7.55 – 7.86 | 27 – 28 |
| Initial | NA | NA | NA | NA |

Table B

| Test concentration (ng/L) | 24 hour # Dead/ # Exposed | 48 hour # Dead/ # Exposed | 96 hour # Dead/ # Exposed | 7 day # Dead/ # Exposed | 14 day # Dead/ # Exposed | 14 day Dry weight mg |
|---|---|---|---|---|---|---|
| 100 | 0/30 | 5/30 | 22/30 | 29/30 | 30/30 | |
| 32 | 4/29 | 4/29 | 14/29 | 19/29 | 21/29 | 2.67 ± 0.91 |
| 10 | 1/30 | 1/30 | 1/30 | 2/30 | 4/30 | 2.58 ± 1.47 |
| 3.2 | 0/30 | 0/30 | 0/30 | 0/30 | 2/30 | 3.24 ± 1.14 |
| 1 | 2/30 | 2/30 | 2/30 | 2/30 | 3/30 | 3.25 ± 1.79 |
| 0 | 0/30 | 1/30 | 1/30 | 1/30 | 5/30 | 3.40 ± 1.16 |
| Initial | NA | NA | NA | NA | NA | 3.14 ±1.99 |

One replicate of 32 ng/L was tipped so only 29 replicates

Table C

| Nominal Test concentration (ng/L) | Measured test concentration (ng/L) t=3 days samples before renewal | Measured test concentration (ng/L) t=3 days samples after renewal |
|---|---|---|
| 100 | 13.6 | 60.0 |
| 10 | 1.92 | 8.21 |
| 1 | Interference | 1.47 |
| 0 | | <0.33 |